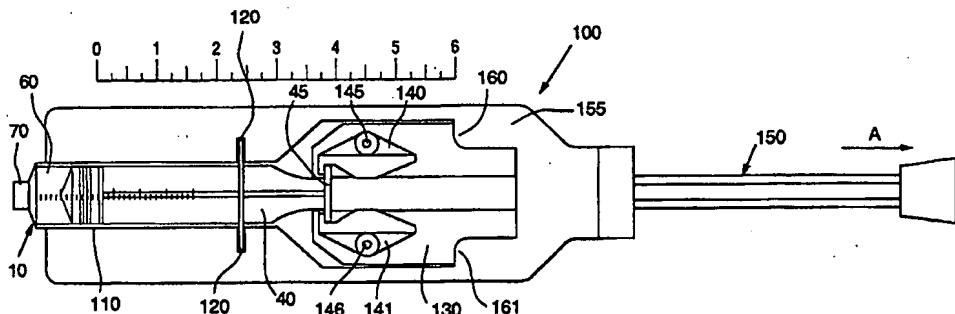




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 49/00		A2	(11) International Publication Number: WO 96/40282
			(43) International Publication Date: 19 December 1996 (19.12.96)
(21) International Application Number: PCT/US96/09108		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 6 June 1996 (06.06.96)			
(30) Priority Data: 08/477,462 7 June 1995 (07.06.95) US			
(71) Applicant: SONUS PHARMACEUTICALS, INC. [US/US]; Suite 102, 22026 20th Avenue, S.E., Bothell, WA 98021 (US).			
(72) Inventors: QUAY, Steven, C.; Suite F-454, Highway 99, Edmonds, WA 98026 (US). KESSLER, Dean, R.; 17318 72nd Street W., Edmonds, WA 98026 (US). ROY, Ronald, A.; 11305 117th Place N.E., Kirkland, WA 98033 (US). WORAH, Dilip; 17200 105th Avenue N.E., Bothell, WA 98021 (US).			
(74) Agents: BENGTTSSON, W., Patrick et al.; Limbach & Limbach L.L.P., 2001 Ferry Building, San Francisco, CA 94111-4262 (US).		<p>Published</p> <p><i>Without international search report and to be republished upon receipt of that report.</i></p>	

(54) Title: NUCLEATION AND ACTIVATION TECHNIQUES FOR USE IN ULTRASOUND IMAGING



(57) Abstract

A method of activating an ultrasound contrast agent such that an increased number of microbubbles are produced and subsequent ultrasound images using the activated agent are enhanced. Hypobaric methods are disclosed which provide significant increases in bubble population per unit volume over unactivated solutions. The method is particularly useful for the activation of the dispersed phase in a gas or liquid in liquid emulsion.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

NUCLEATION AND ACTIVATION TECHNIQUES FOR
USE IN ULTRASOUND IMAGING

5

RELATED APPLICATION

This application is a continuation-in-part application of pending U.S. Patent Application Serial No. 08/477,462 filed June 7, 1995, the contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

This invention relates to methods for nucleating gas-in-liquid or liquid-in-liquid colloidal dispersions or emulsions of microbubble precursors for more effective use in ultrasound imaging. It also relates to activation of preexisting microbubbles in solutions for more effective use in ultrasound imaging. More specifically, the invention concerns methods by which the formation of gaseous microbubbles is promoted in such dispersions prior to or during their introduction into the animal being imaged.

BACKGROUND OF THE INVENTION

Various contrast agents for use in diagnostic ultrasound, including echocardiography, have been described. A review of the subject is found in Ophir and Parker, Ultrasound in Med. & Biol. (1989), 15:319-333, although research has intensified since its publication.

In more recent years, agents which include gaseous microbubbles or microbubble precursors (as opposed to completely solid or liquid agents) have become the subject of great interest, since these

agents take advantage of the relatively high echogenicity of gases versus liquids or solids.

To enhance the performance of microbubbles as ultrasound contrast agents, numerous approaches have been disclosed. These approaches are generally directed to methods by which either the persistence of the bubbles is increased or the bubble size population is optimized.

For example, such approaches include the use of solid particulates into which the bubbles are infused before use (e.g. U.S. 5,147,631), and the use of encapsulants or stabilizers for the microbubbles (such as human serum albumin, e.g. U.S. 4,718,433), each of which is incorporated by reference herein.

Another approach has been to identify compounds which, as gases, are relatively more persistent in blood than air (e.g. U.S. Pat. Nos. 5,393,524 and 5,409,688 and U.S. patent application S.N. 08/380,085, having the same assignee as the present application), and the use of a select number of such gases which are normally liquids at manufacturing temperatures but gases in the body in liquid-in-liquid dispersions (e.g. U.S. patent applications S.N. 08/008,172, S.N. 08/148,284 and S.N. 08/182,024, also assigned to the assignee of the present application) all of which are hereby incorporated by reference.

One method of producing microbubbles in a solution for use with ultrasound imaging is to disperse a gas in a liquid according to U.S. Pat. No. 4,832,941 by manual suspension, e.g., by spraying the liquid backwards and forwards in the gas atmosphere 25 times via a three-way tap.

Also, the use of ultrasound energy to promote the formation of microbubbles for use in ultrasound contrast is known both from U.S. 4,572,203 to

Feinstein (prior to administration) and Molecular Biosystem's WO 94/28939 (in vivo, after administration), both of which are incorporated by reference herein. Neither of these methods, however, produce microbubbles capable of visualizing myocardial perfusion with a peripheral injection.

While such approaches have improved the quality and usefulness of diagnostic ultrasound contrast, further enhancements which provide even better images are desirable.

SUMMARY OF THE INVENTION

To meet this need, this invention is directed to a hypobaric method of activating a gas-in-liquid dispersion, a liquid-in-liquid dispersion, or a mixed gas-liquid-in-liquid dispersion (such dispersions may also contain additional carriers or stabilizers), so that greater numbers of bubbles are formed in a desired size range. Further, the invention relates to methods of ultrasound imaging which include the activating process of the invention prior to administration of the agent. The methods of the invention surprisingly provide improved bubble populations and stability over prior methods.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a syringe for use in an activation unit according to the present invention.

Figure 2 shows a top view of one embodiment of an activation unit according to the present invention.

Figure 3A shows a top view of another embodiment of an activation unit according to the present invention.

Figure 3B shows a side view of the activation unit shown in Figure 3B.

Figure 3C shows a detailed top view of a part of the activation unit shown in Figure 3A.

DETAILED DESCRIPTION OF THE INVENTION

5 The invention will best be understood by the description of specific embodiments, keeping in mind that the claimed invention is not to be construed as limited to the examples described here. As will be understood from these examples, the activation process of the invention provides enhancement of the contrast in an ultrasound image generated during medical or veterinary diagnosis.

Definitions

To ensure a complete understanding of the invention the following definitions are provided:

15 Surfactants: The group of amphiphilic materials which are manufactured by chemical processes or purified from natural sources or processes. These can be anionic, cationic, nonionic, and zwitterionic. Such materials are described in Emulsions: Theory and Practice, Paul Becher, Robert E. Krieger Publishing, 20 Malabar, Florida, 1965 which is hereby incorporated by reference.

Amphiphilic Material: A substance which is strongly adsorbed at an interface and which normally produces a dramatic reduction in the interfacial tension with small changes in the bulk phase concentration. Examples include synthetic surfactants, naturally occurring materials such as biocompatible proteins, lipids, sterols, alginates, 25 cellulose derivatives, and finely divided organic or inorganic particulate solids.

Polyoxypropylene-Polyoxyethylene Glycol Nonionic Block Copolymers: The surfactants which are available from BASF Performance Chemicals, Parsippany, New 30

Jersey under the trade name Pluronic and which consists of the group of surfactants designated by the CTFA name of poloxamer 108, 188, 217, 237, 238, 288, 338, 407, 101, 105, 122, 123, 124, 181, 182, 183, 184, 212, 231, 282, 331, 401, 402, 185, 215, 234, 235, 284, 333, 334, 335, and 403.

Fluorine-Containing Surfactant: A surfactant containing one or more fluorine atoms. Some, but not necessarily all, fluorine containing surfactants useful in this invention can be selected from the group consisting of: telomer B containing fluorinated surfactants available from Du Pont, Wilmington, DE under the Trade name of Zonyl (including Zonyl FSA, FSP, FSE, UR, FSJ, FSN, FSO, FSC, FSK, and TBS), the fluorochemical surfactants from 3M Industrial Chemical Products Division, St. Paul, MN under the trade name of Fluorad (including FC-95, FC-98, FC-143, FC-170C, FC-171, FC-430, FC-99, FC-100, FC-120, FC-129, FC-135, FC-431, FC-740), the perfluoroalkylpoly(oxyethylene) surfactants described by Mathis *et al.* (*J Am Chem Soc* 106, 6162-6171 (1984), incorporated herein by reference), the fluoroalkylthio-etherpoly(oxyethylene) surfactants described by Serratrice *et al.* (*J Chim Phys* 87, 1969-1980 (1990), incorporated herein by reference), the perfluoroalkylated polyhydroxylated surfactants of Zarif *et al.* (*J Am Oil Chem Soc* 66, 1515-1523 (1989), incorporated herein by reference), the fluorosurfactants available from Atochem North America, Philadelphia, PA under the trade name of Forafac.

Activation: A method step by which bubble formation, specifically microbubble formation, is encouraged or promoted when that step is applied to a solution which is to be administered as an ultrasound contrast agent. More specifically, activation within

the meaning of the invention refers to the application of a hypobaric force on a solution which contains microbubbles or microbubble precursors to cause microbubble production, size change or additional microbubbles of gas to form in said solution.

Microbubble Precursor: a microbubble nuclei, i.e., any solid or liquid particle or gaseous macrobubble which is capable of producing microbubbles of a gas contained therein under the conditions referred to herein as activation conditions.

Colloidal Dispersion: A system having at least one substance as a solid, liquid or gas (the dispersed phase) which is immiscible and finely divided and distributed evenly throughout at least one second substance which forms the dispersion medium or continuous liquid phase.

Biocompatible: Capable of performing functions within or upon a living organism in an acceptable manner, without undue toxicity or physiological or pharmacological effects.

High Vapor Pressure Chemical: A chemical with a sufficiently high vapor pressure that colloidal dispersions of the chemical as a liquid contain, at the body temperature of an organism undergoing an ultrasound examination, a sufficient quantity of the chemical as a gaseous dispersion to provide a diagnostically useful alteration in the ultrasound data obtained during an examination. High vapor pressure chemicals include low boiling liquids, a preferred embodiment of the present invention. Chemicals with vapor pressures at ambient temperature of above 20 Torr (typically having boiling points below 135°C) are another preferred embodiment. The latter class of chemicals which are useful in this

invention include, but are not limited to:
perfluorinated n-alkanes, cycloalkanes, and branched
alkyl compounds containing up to nine carbons
(nonane; B.P. 125°C); alkyl hydrocarbons containing
up to ten carbons, such as n-decane; ethers, other
organic halides, and alcohols.

Fluorine-Containing Compounds: A compound
containing at least one fluorine atom.

Emulsion: A colloidal dispersion of one
immiscible liquid dispersed in another liquid in the
form of droplets, whose diameter, in general, are
between 100 and 3000 nm and which is typically
optically opaque, unless the dispersed and continuous
phases are refractive index matched. Such systems
possess a limited stability, generally defined by the
application or relevant reference system, which may
be enhanced by the addition of amphiphilic materials
or viscosity enhancers.

Microemulsion: A stable liquid monophasic and
optically isotropic colloidal dispersion of water and
water-immiscible liquids stabilized by amphiphilic
materials in which the dispersions have appreciable
light scattering properties (meaning they can appear
optically clear or milky but are reddish or yellowish
if observed by transmitted light) and the diameters
of the particles are, in general, between 5 and
approximately 140 nm.

Organic Particulate Solids: include sugars,
proteins, amino acids, lipids, nucleic acids, and
others.

Inorganic Particulate Solids: include aluminas,
carbonates, bicarbonates, silicates,
aluminasilicates, phosphates, and others.

Aqueous Medium: A water-containing liquid which
can contain pharmaceutically acceptable additives
such as acidifying agents, alkalizing agents,

antimicrobial preservatives, antioxidants, buffering agents, chelating agents, complexing agents, solubilizing agents, humectants, solvents, suspending and/or viscosity-increasing agents, tonicity agents, wetting agents or other biocompatible materials.

Agents With Which The Method May Be Used

The activation method of the invention can be used on any emulsion, dispersion, or liquid (e.g. gas-in-liquid, liquid-in-liquid and surfactant containing liquids) in which it is desired that bubble formation be promoted or enhanced. Further, there may be instances when the activation method can be used to advantage when the solution on which the activation step is performed would not strictly be considered a dispersion or emulsion, but when bubble activation will nevertheless advantageously occur. For example, agents which incorporate a solid carrier, whether organic or inorganic, can be advantageously subjected to activation according to the invention. Additionally, solutions which contain stabilizers (such as amphiphilic material including human serum albumin or surfactant solutions), which might not strictly be considered dispersions, can likewise be advantageously activated by the invention.

Gases which may be the subject of activation will be selected from those already known in ultrasound contrast, keeping in mind that biocompatible compounds which are more persistent are preferred. Thus, gases of fluorine-containing chemicals can especially be used.

The activation process is particularly advantageous when used with gas or liquid-in-liquid emulsions or dispersions. Expected to be of particular value are those dispersions described in

applications S.N. 08/008,172, S.N. 08/148,284 and
S.N. 08/182,024, each of which is incorporated herein
by reference, in which the dispersed phase is liquid
at common processing and storage temperatures, but
5 which forms a gas, at least in part, at the body
temperature of the animal in which the agent will be
used.

The activation process is also advantageous when
used with the dispersions described in applications
10 PCT WO 95/03835, PCT WO 94/28939, WO 94/28797, WO
94/28780, and U.S. Pat. No. 4,832,941, each of which
is incorporated herein by reference.

In a further preferred embodiment, the process
will be applied to liquid-in-liquid colloidal
15 dispersions in which the continuous phase is aqueous
and the dispersed phase is a fluorine containing,
branched or linear, hydrocarbon. In this class, the
most preferred dispersed phase compounds will be C₁-C₈
fluorine substituted hydrocarbons, due to their
20 biocompatibility, and the fact that they have boiling
points below the body temperatures of animals of
interest and/or have vapor pressures high enough
(even if they boil above such temperatures) to
provide microbubbles in quantities sufficient to
25 enhance ultrasound contrast in use.

Even more particularly preferred are emulsions
in which the dispersed phase is selected from one or
a mixture of the following: sulfur hexafluoride;
tetrafluoromethane; perfluoroethane;
30 perfluoropropane; perfluorobutane; perfluoropentane;
perfluorohexane; and perfluoroheptane and
perfluorooctane and mixtures or derivatives thereof.

Activation Method

In order to provide enhanced ultrasound
35 contrast, the activation method will provide a means

by which bubbles of a desired size population are formed prior to administration of the bubbles in the body. Methods of activation according to the invention include approaches which may be considered
5 under a general category of the application of "hypobaric" forces to the solution in question.

Hypobaric forces are to be distinguished from hydrodynamic or acoustic under standard definitions.

"Hydrodynamic" forces, which can be single pass
10 or multiple pass, are those forces that operate during the generation of bulk motion of fluids. The methods described in U.S. Pat. No. 4,832,941 are an example of such forces.

"Acoustic" forces are those that operate during
15 the propagation of sound waves in a fluid. Generally, continuous or intermittent acoustic energy is applied rather than a single acoustic wave.

"Hypobaric" forces are those that operate during the reduction of hydrostatic pressure in a fluid,
20 result in isotropic stresses, and do not necessarily involve shearing motion, or propagating acoustic fluctuations.

Encompassed by the class of hypobaric forces of the invention are the forces created when the
25 solution is forced rapidly through a filter or when the pressure in the solution is rapidly lowered to below atmospheric by placing the solution in a sealed vessel and rapidly lowering the pressure in the vessel. This is in distinction to the methods in
30 which liquids are stored under higher pressure (above atmospheric) which are released to expose the liquid to atmospheric, not hypobaric, pressures, for example see U.S. 5,334,381.

The invention will be better understood by way
35 of the following examples; however, it is to be understood that these examples are illustrative only

and are not to be construed as limiting the invention as claimed below:

Example 1. A preferred method of hypobaric activation

5 A 2.0, 3.0 or 4.0 mL volume of EchoGen® emulsion (Sonus Pharmaceuticals, Bothell, Washington), a 2% perfluoropentane emulsion described in S.N. 08/182,024, was administered intravenously following activation in a patient via an antecubital vein. All
10 solutions of 2% EchoGen® emulsion were injected using sterile syringes using aseptic technique.

Method of Administration

Materials needed:

15 2% EchoGen® emulsion
 Saline for injection
 3-way stopcock
 10 or 20 mL luer-lock polycarbonate syringe (Merit Medical Systems, South Jordan, Utah)
 18 gauge needle
20 20 gauge angiocath

1. Determine the volume of 2% EchoGen® to be administered.

• If \leq 2mL use a 10 mL syringe with luer-lock for administration

25 • If $>$ 2mL use a 20 mL syringe with luer-lock for administration

2. Connect a 3 way stopcock and 18 gauge needle to the appropriate size syringe.

3. Withdraw the calculated volume and 1 mL
30 additional of 2% EchoGen® emulsion from the vial.

4. Clear the syringe of air and EchoGen® to achieve the correct administration dosage and turn the stopcock to the closed position to seal the syringe. Remove the needle.

35 5. Connect a 20cc saline flush syringe to the 90° port on the 3 way stopcock. Push saline to expel air from the stopcock port.

- 5 6. Holding the EchoGen® Emulsion containing syringe by the barrel in a horizontal position, rapidly pull back on the syringe plunger to the extent of the graduations on the syringe (to the 10 mL or 20 mL mark) and immediately release the plunger. This should result in an audible "pop". This audible "pop" is the result of the plunger exciting an acoustic pressure wave in the air behind the advancing plunger. It is not an acoustic mode in the contents of the syringe.
- 10 7. Immediately (within 5 seconds) attach the syringe assembly via the stopcock onto the 20 gauge Angiocath located in the patient's antecubital vein.
- 15 8. Turn the stopcock to allow EchoGen® emulsion administration and inject.
9. Turn the stopcock to the saline flush syringe and inject 10 mL.

A 0.05 mL/kg dose produced myocardial perfusion in a human subject.

20 Example 2. Comparison of hypobaric and non-hypobaric activation

The objective of this study was to evaluate the hemodynamic parameters and ultrasound imaging effects of EchoGen® 2% Emulsion with a hypobaric pre-activation process following intravenous administration to an anesthetized monkey.

25 A monkey was anesthetized with ketamine intravenously, intubated, and allowed to spontaneously breathe room air. End tidal CO₂ was monitored continuously. A catheter was placed in the femoral artery to monitor blood pressure and to obtain arterial blood samples for blood gas analysis.

30 A 5 French catheter was inserted into the right external jugular vein and advanced into the pulmonary artery for monitoring pulmonary artery pressure. In

35

addition, ECG leads (lead II), a rectal temperature probe and pulse oximeter were attached to the monkey for additional monitoring.

5 EchoGen® 2% Emulsion was administered via a 20 gauge angiocath inserted into the femoral vein. Peripheral venous injections of EchoGen® were performed following hypobaric activation within the syringe at dosages of 0.05 and 0.1 mL/kg bodyweight. The following parameters were continuously monitored and recorded at designated time points before and after administration: electrocardiograms (lead II), 10 systemic blood pressure (systolic, diastolic, and mean), heart rate, pulmonary artery pressure (systolic, diastolic and mean), end-tidal CO₂, O₂-saturation, and respiratory rate. Ultrasound imaging was performed before, during and after each injection with an ATL Ultramark 9 with a P7-4 transducer. Images were recorded on SVHS videotape.

20 The monkey received 9 bolus intravenous administrations of EchoGen® 2% Emulsion at dosages of 0.05 or 0.1 mL/kg at approximately 30 minute intervals. A total of 9 administrations during the study led to a potential maximum cumulative dosage of 7.5 mL or 0.7 mL/kg. At the end of the monitoring 25 period, the animal was euthanized by an intravenous injection of Beuthanasia-D®.

30 There were no adverse clinical signs or test article-related effects on blood gas parameters, arterial pH, electrocardiograms, respiratory rate, end-tidal CO₂, O₂-saturation or pulmonary artery pressure.

35 There were minor, transient test article-related effects on systemic blood pressure. The general effect was a decrease in systolic, diastolic, and mean blood pressures (10-18%) within the first minute following administration of EchoGen® 2% Emulsion

which returned to baseline spontaneously within 90 seconds.

5 Injections at all dosages produced dense right and left cardiac chamber opacification without excessive attenuation of the signal. Following pulmonary transmission, dense myocardial enhancement was observed at dosages of 0.05 and 0.1 mL/kg bodyweight. Ventricular contractions were essentially unchanged and alterations in arterial
10 pressure were transient.

 Using non-hypobaric activation through a filter the same level of myocardial enhancement required a dose of 0.6 mL/kg or greater, up to 10-times the above dose.

15 Under the conditions of the study, dosages of 0.05 and 0.1 mL/kg of EchoGen® 2% Emulsion administered intravenously following hypobaric pre-activation had no significant effect on the cardiovascular parameters measured in an anesthetized
20 monkey expect for a transient, minor decrease in mean systemic arterial blood pressure. The transient decreases in systemic arterial blood pressure (10-18%) returned to baseline within 90 seconds. The hypobaric pre-activation method provided a dramatic
25 improvement in efficacy as evidenced by the dense myocardial enhancement observed at a dosage of 0.05 and 0.1 mL/kg.

 In conclusion, the method of hypobaric activation of EchoGen® 2% Emulsion immediately prior
30 to administration is significantly more efficacious for providing dense ultrasound contrast enhancement without significant adverse hemodynamic effects.

Example 3. Comparison of the Activation Methods for EchoGen® Emulsion, 2% DDFP: *in vitro* Bubble Size
35 Analysis

The objectives of these experiments were to use bubble size and density measurements using phase Doppler light scattering interferometry to compare activation of EchoGen® Emulsion, *in vitro*, sonication and hypobaric methods.

We have shown that activation (converting the liquid droplets to microbubbles) prior to administration provides greater efficacy and could permit the use of lower doses. Three methods of activation were compared:

1. Hydrodynamic Filtration: By passing the emulsion droplets through a 1.2 μm filter under pressure, the emulsion will experience a barometric pressure decrease;

2. Sonication; and

3. Hypobaric Sealed Container Method: By placing the emulsion in a sealed syringe and vigorously withdrawing the plunger and then releasing the plunger, one can lower the pressure in the liquid and activate the liquid droplets.

Few methods exist for the determination of the size of gas bubbles. In these experiments the Phase Doppler Interferometric (Aerometrics, Inc., Mountain View, CA) method of particle sizing was used. This method uses the phase shift of the light transmitted through or reflected from spherical particles and collected at large off axis angles.

The laser phase Doppler particle sizer determines number density using Beer's law for particles of approximately 10 μm in size, the maximum number density is $10^7/\text{cm}^3$. When this concentration is exceeded, the instrument cannot obtain reliable data concerning number density or particle size, (this phenomena is similar to attenuation in ultrasound). Bubble concentration from all activation methods exceeded this value when the undiluted drug was

activated. Since the activation methods differed in the degree to which the drug was activated by a wide margin, the concentration of each had to be adjusted individually in order to obtain accurate measurement of bubble size and density. Bubble size was determined by activating a small quantity of the drug, 0.1 to 1 mL, diluting it to proper concentration with saline, and delivering it to the cell, (5 x 1 cm with the sides attached at 72° and 108° angles respectively) where it was isolated by closing the stopcock placed above and pinch clamp placed directly below. The cell was aligned so that the receiving cell was 108° from the sending laser. For particles in the 1-35 μ m range, data was obtained every 10 seconds for 1-2 minutes. The data from the first 30 seconds was combined for better statistical relevancy.

Activation Procedures

1. Filtration

Filtration through a 1.2 μ m filter. One mL of EchoGen® Emulsion was filtered through a 1.2 μ m Acrodisc filter (25 mm flat disc) into 4 mL of saline and the entire mixture (5 mL) was introduced into the cell at the rate of 0.5 mL/s.

2. Sonication.

One tenth of 1 mL (0.1mL) of EchoGen® Emulsion was sonicated at 42 KHz in a 5 mL tubing for 10 seconds with 10 second lag phase following which the material was introduced into the cell with 6 mL saline at the rate of 0.5 mL/s.

3. Hypobaric Activation.

One mL was of EchoGen® Emulsion withdrawn into a 10 mL syringe equipped with a 3-way stopcock. The stopcock was set so the syringe barrel was closed. The plunger was rapidly pulled back to the 10 mL mark

and allowed to snap back. One tenth of 1 mL (0.1mL) of the material was introduced into the cell with 6 mL saline at the rate of 0.5 mL/s.

RESULTS

5 The data for the first 30 seconds for the various methods was averaged and is presented in Table 1.

Table 1
Summary of Bubble Measurements

10	Activation Method	Volume of EchoGen®	Normalized Mean diameter	Number of Bubbles/mL of EchoGen® Emulsion x10 ⁶
	None	1	None	<0.1
	1.2 μ m Filtration	1	100%	6.8
15	Sonication	0.1	98%	144
	Hypobaric Activation	0.1	96%	78

20 * The actual measured diameters, which are precise but not necessarily accurate, were normalized to the value obtained with the 1.2 μ m filter

25 All three methods of activation result in approximately equal microbubble sizes, but the number of particles activated into microbubbles differ considerably. Sonication and hypobaric methods generate approximately 21 and 11 times more bubbles, respectively, than the filtration method and all three methods were superior to unactivated EchoGen® emulsion.

Example 4. A preferred hypobaric sealed container construction.

5 In the Hypobaric Sealed Container Method of activation, the solution to be activated is placed in a sealed syringe and the pressure is then reduced by rapidly withdrawing the plunger. The plunger is then immediately released and snaps back to its initial position. This last step generates large pressures in the syringe, 40 to 60 atmospheres, and this may
10 cause problems. In fact, using conventional glass or plastic syringes in this activation method resulted in about a 20% breakage rate of the syringe body. For this reason, it was realized that the sealed container used in the Hypobaric Sealed Container
15 Method of activation must be made of a strain-resistant material. Specifically, the sealed container must be capable of withstanding pressures of at least 40 to 60 atmospheres. Furthermore, the sealed container must be capable of withstanding a
20 pressure change of about 40 to 60 atmospheres occurring over a fraction of a second--the time it takes the plunger to snap back into position. In one embodiment, the sealed container is made of polycarbonate resin. In a preferred embodiment, the
25 sealed container is a polycarbonate syringe, i.e. a syringe with a polycarbonate resin body. For example, Medallion[™] brand syringes manufactured by Merit Medical Systems, South Jordan, Utah 84095 may be used. In over 500 trials using these syringes
30 there have been no breakages of the polycarbonate syringe bodies.

Example 5. A preferred device for hypobaric activation.

Figures 1-3 show a preferred device for achieving hypobaric activation according to the present invention.

5 Figure 1 shows a standard syringe 10 for use in the present invention. The syringe includes a syringe body 20 having a flange 30, a syringe plunger 40 having a flange 45, and a rubber stopper 50 attached to syringe plunger 40. The solution to be activated is contained in cavity 60. The syringe is
10 filled and discharged through opening 70. As discussed above, it is preferred that the syringe is made of a polycarbonate resin.

Figures 2 and 3A-3C show syringe 10 mounted on a hypobaric activation unit 100. The activation unit
15 100 includes the following elements:

- a syringe stabilizer 110 and slots 120 for holding the syringe body 20 and flanges 30;
- a movable plate 130 attached to a handle 150;
- hooks 140 and 141 mounted on plate 130 and
20 pivoted about pivots 145 and 146, for securing syringe plunger flange 45;
- a frame 155 having shoulders 160 and 161;
- a base 170 to which the syringe stabilizer 110 and frame 155 are fixed and which supports movable
25 plate 130; and
- a spring 180 attached to the base 170 and movable plate 130.

To prepare an activated dose of contrast agent, the operator first places the syringe 10 in the
30 activation unit 100 so that the syringe body 20 is positioned in syringe stabilizer 110 and flanges 30 are positioned in slots 120. The syringe plunger 40 is positioned so that flange 45 is secured by hooks 140 and 141.

35 After positioning the syringe in the activation unit, the operator fills the syringe with a

predetermined amount of the solution to be activated. To achieve the syringe filling step, the operator first connects syringe opening 70 to a supply of the solution to be activated (not shown). The operator
5 then withdraws handle 150 a predetermined amount in direction A shown in Figure 2. Handle 150 is attached to movable plate 130 which supports hooks 140 and 141. Withdrawing handle 150 in direction A therefore moves hooks 140 and 141 in direction A and
10 this in turn withdraws syringe plunger 40 from syringe body 20 which is restrained from moving by flanges 30 in slots 120. In this way, a predetermined volume of solution is delivered into syringe cavity 60. After filling the syringe, the
15 opening 70 is disconnected from the solution supply and is sealed. Alternatively, the syringe may be filled manually with a predetermined volume of solution prior to being placed in the activation unit. In this case, the above filling step is
20 omitted.

After the syringe has been filled, the solution is activated in the following manner.

First, the operator rapidly withdraws handle 150 in direction A to its fully extended position. This
25 has the effect of rapidly withdrawing syringe plunger 40 from syringe body 20 which reduces the pressure of the solution contained in cavity 60. When handle 150 is withdrawn to its fully extended position, hooks 140 and 141 contact frame shoulders 160 and 161 and
30 this causes the hooks to pivot into their open position thus releasing syringe plunger 40. Figure 3C shows the hooks in their open position. When the hooks release the syringe plunger, the reduced pressure in the solution causes the plunger to "pop"
35 back into its unextended position. At this time the

solution is activated and is ready to be injected into the subject.

5 The design of the activation unit shown in Figures 2 and 3 ensures that the syringe plunger is always released after it has been withdrawn to a predetermined extension. This feature allows the operator to control the hypobaric conditions used in activating the solution in a precise and consistent manner, thus minimizing operator error and increasing
10 the consistency of the properties of the activated contrast agent.

 After the hooks have released the plunger, the operator releases handle 150 and spring 180 then returns plate 130 and hooks 140 and 141 to their
15 starting position ready to receive the next syringe.

What is claimed is:

1. A method of ultrasound imaging comprising the steps of:

5 applying hypobaric forces to a solution
containing a gas or gas precursor to form a
microbubble-containing solution;
 administering said microbubble-containing
solution to an animal; and
10 performing an ultrasound scan on a portion of
said animal into which said solution has passed.

2. A method of ultrasound imaging according to claim 1 wherein said hypobaric force is generated by reducing the hydrostatic pressure within said solution in sealed container.

15 3. A method of ultrasound imaging according to claim 1 wherein said hypobaric force is generated by forcing said solution through a filter.

 4. A method of ultrasound imaging according to claim 3, wherein said filter has openings of from
20 about 0.8 to about 10 microns.

 5. A method of ultrasound imaging according to claim 3 wherein said filter has openings of approximately 1.2 μm .

25 6. A method of ultrasound imaging comprising the steps of:
 placing a liquid dispersion in a sealed
container, said dispersion containing a liquid or gas
dispersed phase comprising a high vapor pressure
30 chemical;

lowering the pressure in said container to a pressure adequate to cause the formation of additional gas microbubbles containing said chemical; administering said microbubble-containing solution to an animal; and performing an ultrasound scan on a portion of said animal into which said solution has passed.

7. The method of claim 6, wherein said dispersed phase chemical is selected from the group consisting of fluorine containing C₃-C₆ hydrocarbons.

8. The method of claim 6 wherein said gas or gas precursor is a fluorine-containing chemical.

9. An apparatus for applying hypobaric forces to a solution containing a gas or gas precursor to form a microbubble-containing solution, the apparatus comprising a sealed container for containing a solution containing a gas or gas precursor, and means for lowering the pressure in the container to cause gas microbubbles to form.

10. The apparatus of claim 9, wherein the sealed container is made of a strain-resistant material.

11. The apparatus of claim 10, wherein the sealed container is made of polycarbonate resin.

12. The apparatus of claim 9, wherein the sealed container is a polycarbonate resin syringe which includes a syringe body and a plunger, and wherein the means for lowering the pressure is the syringe plunger.

13. The apparatus of claim 12 further comprising means for withdrawing the plunger a predetermined amount sufficient to cause gas microbubbles to form in the solution.

1/2

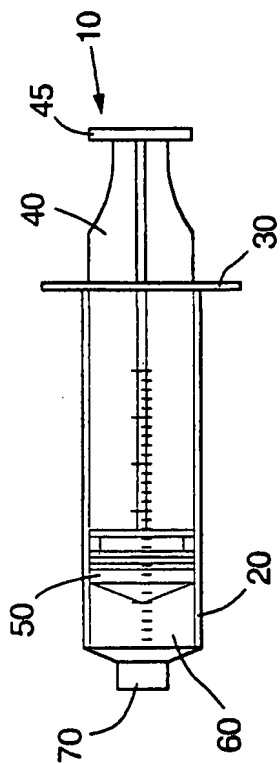


FIG. 1

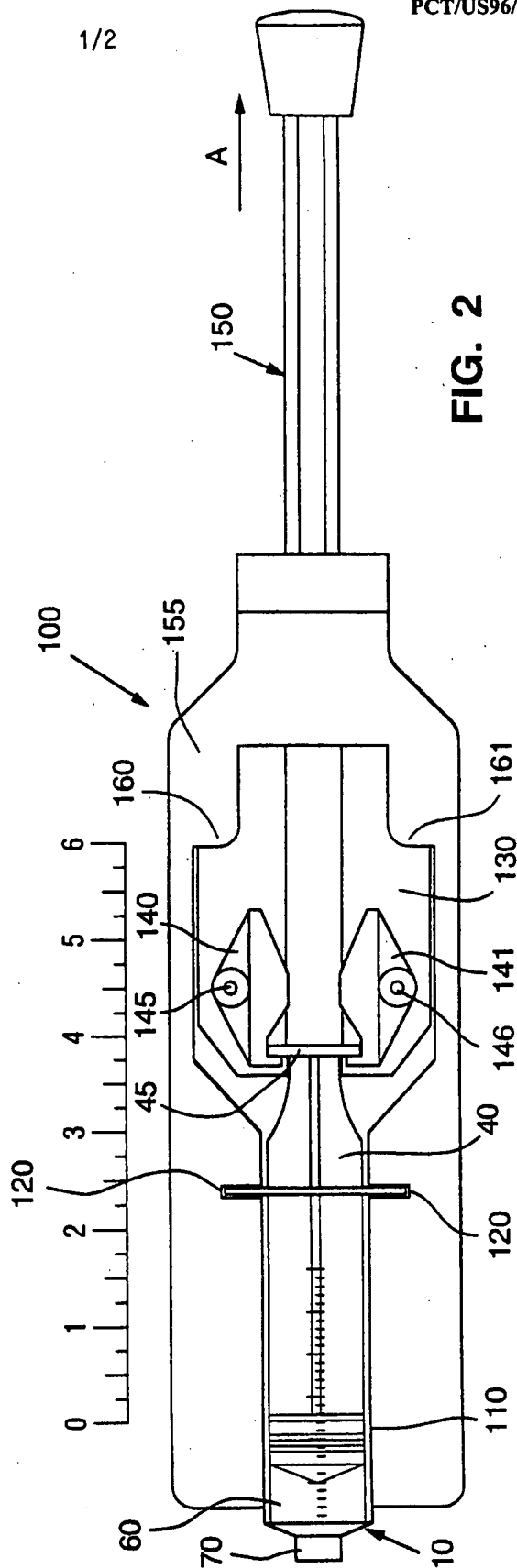


FIG. 2

SUBSTITUTE SHEET (RULE 26)

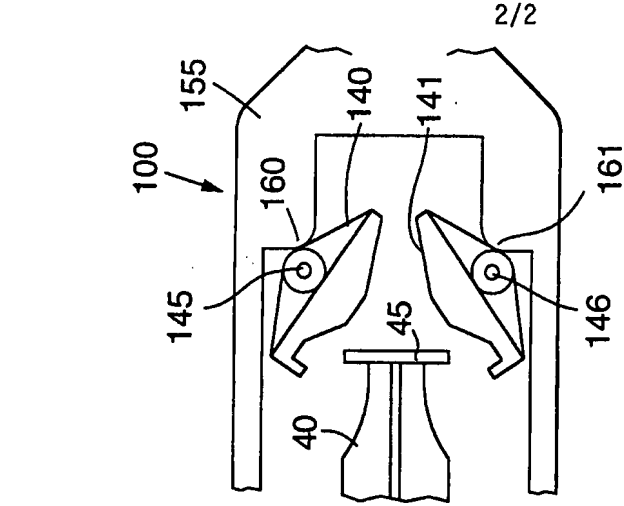


FIG. 3C

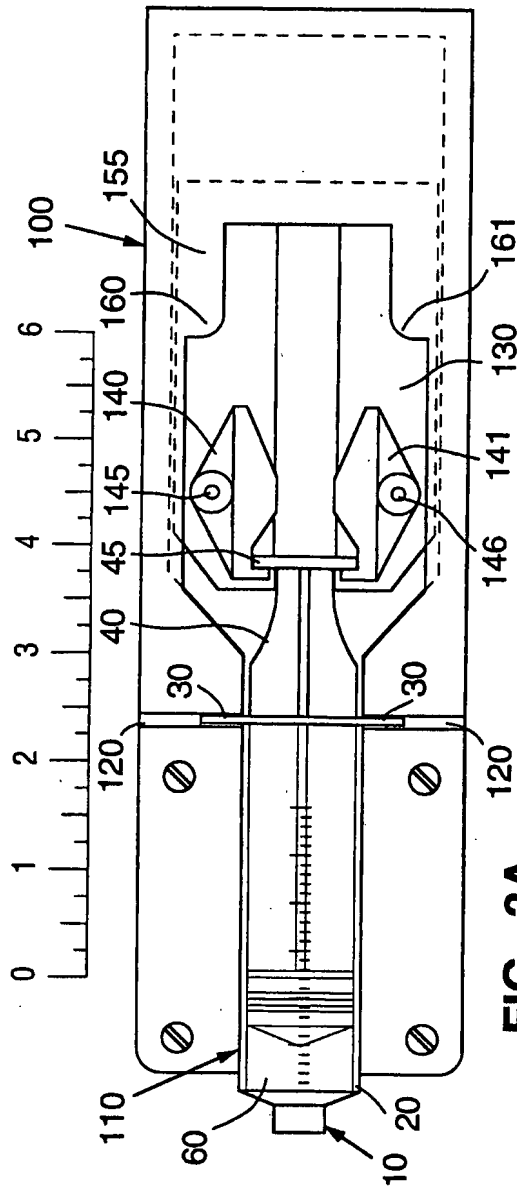


FIG. 3A

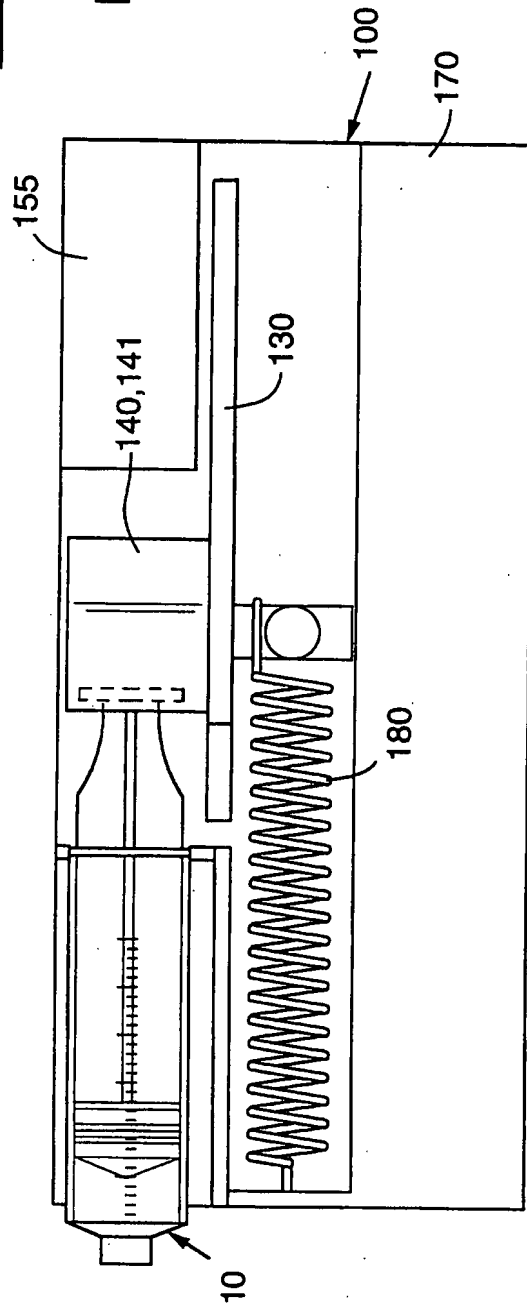


FIG. 3B

SUBSTITUTE SHEET (RULE 26)